The Amsterdam Cohort Studies on HIV infection
Annual Report 2006

Introduction

The Amsterdam Cohort Study (ACS) on Human Immunodeficiency Virus (HIV) infection and AIDS amongst homosexual men was initiated in 1984, followed shortly by the Amsterdam Cohort Study amongst drug users in 1985. The ACS, a collaboration of the Amsterdam Health Service (AHS), the Academic Medical Center of the University of Amsterdam, Sanquin Blood Supply Foundation, and the University Medical Center Utrecht (UMCU), is part of the Netherlands HIV Monitoring Foundation and financially supported by the Netherlands National Institute for Public Health and the Environment.

Thus far, 2299 homosexual men (HM) and 1663 (injecting) drug users (DU) have been included in the ACS. Every 3 to 6 months participants complete a standardized questionnaire designed to obtain information regarding medical history, sexual and/or drug use behaviour, underlying cognitions, health care use, depression, psychological disorders, and demographics. In addition, they undergo a medical examination (HIV-positive participants and, in the past, HIV-negative drug users as well) and blood is drawn for biologic and immunologic tests and storage.

Of the 2299 HM, 571 were HIV-positive at study entry, and 192 seroconverted during follow-up. For the 1663 DU, 323 were HIV-positive at study entry, and 95 seroconverted during follow-up By December 31 2006, 323 HM and 385 DU had died; other participants were requested to leave the study or left at their own request. On average, 90% to 92% of participants who visited the ACS during a given calendar year returned for a follow-up visit the next year. In total, HM visited the Amsterdam Health Service 45,444 times and DU 23,948 times.

Website: http://www.amsterdamcohortstudies.org/

The cohorts in 2006

Homosexual men

In 2006, 536 HM were followed at the Health Service of Amsterdam. Sixty of them were newly recruited in 2006. From 2005, recruitment was open for HM of all ages with at least one sexual partner in the preceding 6 months. Of the HM followed in 2006, 497 men were HIV-negative, and 39 men were HIV-positive. The HIV-positive men, of whom 29 were HIV seroconverters, were followed according to the HIV onderzoek onder positieven (HOP) protocol, which was initiated in October 2003 for HM who seroconverted or were HIV-positive at study entry in the cohort of young HM after 1999. From June 2006, HIV-positive steady partners of HIV-negative participants and all steady partners of HIV-positive participants were also invited to participate in the ACS.
In 2005, 218 HIV-infected HM who were recruited as part of the ACS before 1999 were seen at the Jan van Goyen Clinic or at one of the 22 other HIV treatment centres in the Netherlands. Sixty-two of them were HIV seroconverters. Plasma and cells from HIV-positive HM in active follow-up at the Jan van Goyen Clinic were stored for those who (1) seroconverted during follow-up, (2) had been defined as slow/non progressor or matched fast progressor in 1996, and (3) were HIV-positive for more than 10 years and had a CD4 count greater than 400 cells/mm3 after 10 years of follow-up after a positive HIV result without effective therapy (n=94).

Drug users

In 2006, 472 drug users were followed at the Health Service of Amsterdam; of those, 67 were young drug users aged 30 years or less and recruited after 2000. In 2006, 19 new drug users were included because of the possibility that they received hepatitis C treatment within the cohort setting (the so-called Dutch-C study). The cohort remained open to drug users less than 30 years of age who had used cocaine, heroin, or amphetamines at least 3 times a week in the 2 months preceding enrolment. Sixty-six of the 472 drug users were HIV-infected; of those, 22 seroconverted during follow-up in the ACS.
In 2005, within the DU cohort, a feasibility study was started to evaluate the possibility of hepatitis C virus (HCV) testing and treatment combined with methadone programs. As part of this project (the Dutch-C study), by the end of 2006, 19 HCV mono-infected DU of the cohort had initiated HCV therapy.

HIV incidence

Thirteen homosexual men and no drug users seroconverted for HIV in 2006. Three of them last received a negative HIV test result in 2001, 2004, and 2005, respectively, which implies that the actual moment of HIV infection could have been before 2006. However, we have included these three in the 2006 count. HIV incidence is around 1.8 per 100 person-years amongst HM and less than 1 per 100 person-years amongst DU. Figures 1 and 2 show the yearly HIV incidence rates for homosexual men and drug users since the start of the ACS through 2006.

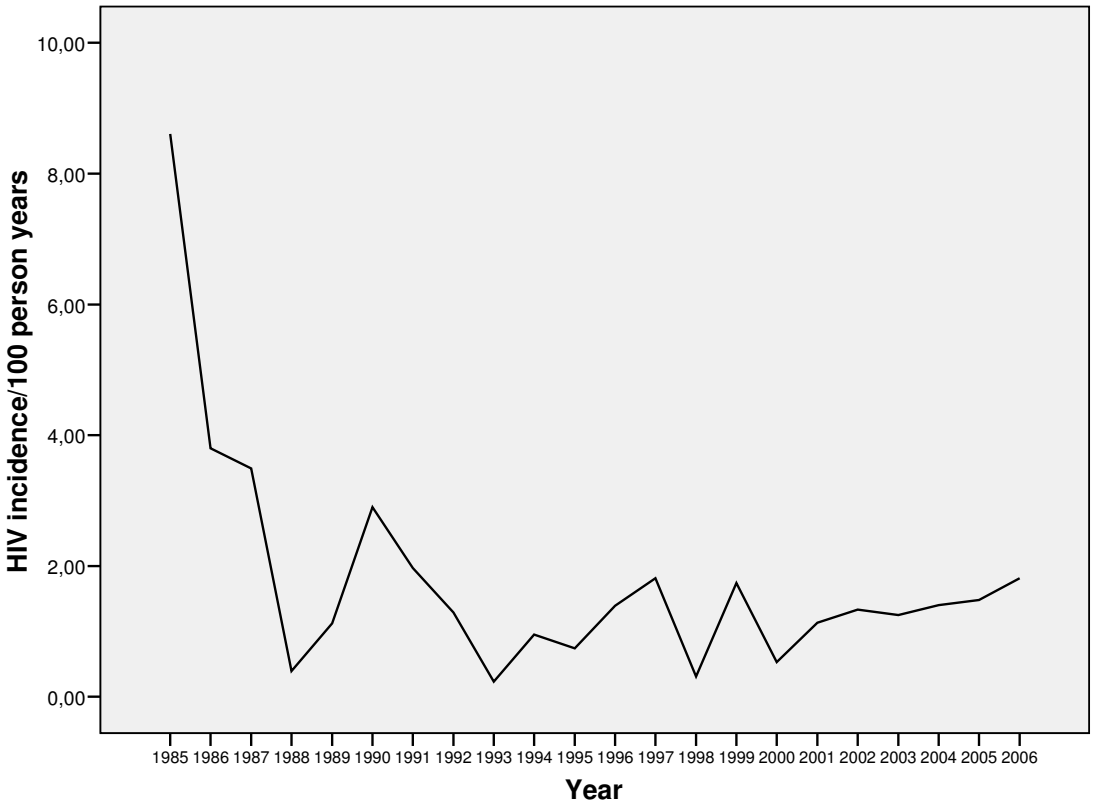

Figure .1. HIV incidence per calendar year in the Amsterdam Cohort Study amongst homosexual men (HM)

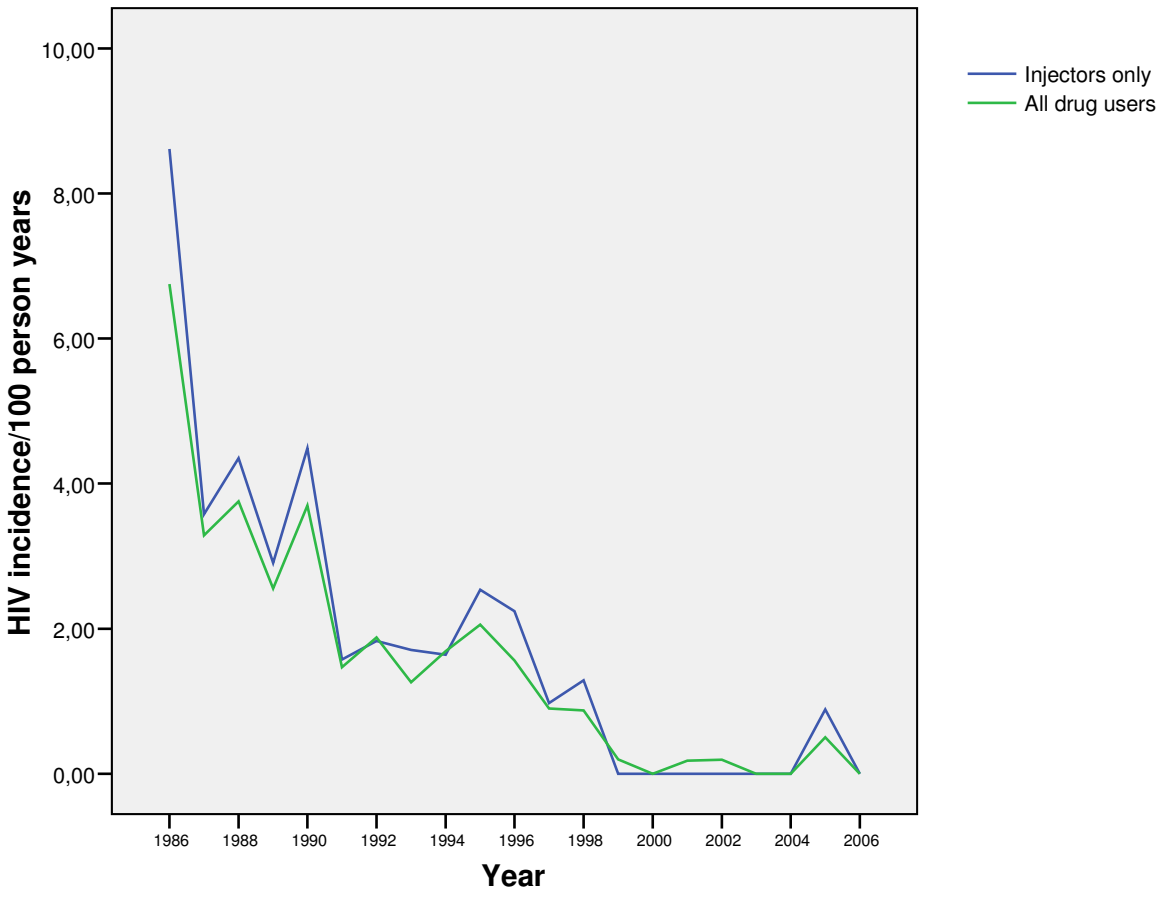

Figure 2. HIV incidence per calendar year in the Amsterdam Cohort Study amongst drug users

Transmission of therapy resistant HIV strains

A total of 100 primary HIV-1 infections (32 AMC hospital and 68 ACS) were identified from 1994-2002. Transmission of drug-resistant mutations decreased over calendar time, with 20% of infection-bearing drug-resistant mutations transmitted before 1998 versus only 6% after 1998 (Bezemer et al, AIDS 2004). In 2005, 2 out of 9 seroconverters within the ACS became infected with a drug-resistant strain. Of 13 HIV seroconverters with a first HIV-positive test result within the ACS in 2006 (all HM), a sequence could be obtained for 12. Of these, one was found to be infected with a drug-resistant strain.

Risk behaviour

In the cohort of HIV-negative HM, trends in the incidence of HIV and sexually transmitted infections (STI) were concurrent amongst young men until 1995. However, since 1995, there has been a significant increase in the incidence of syphilis and gonorrhoea (see Figure 3), but no change in HIV incidence (van der Bij et al, Sex Transm Infect 2005; 81:31-7). In 2006, the 6-monthly questionnaire of the HM was expanded to include questions regarding knowledge and use of post-exposure prophylaxis.

In the cohort of HIV-negative DU, reports of both injecting and borrowing needles significantly declined over the period 1985-2004 (Lindenburg et al, AIDS 2006). Reports of sexual risk behaviour and STI at follow-up visits decreased before 1996, but not after 1996 (see Figure 4).

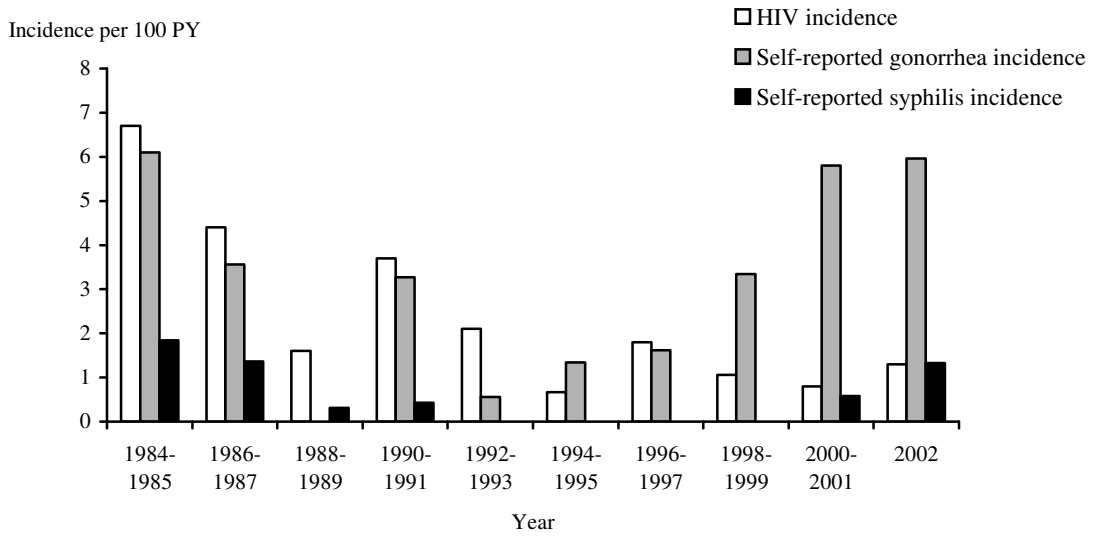

Figure 3. Incidence of gonorrhea, syphilis, and HIV per 100 person-years (PY) amongst 863 HIV-negative young (≤ 30 years at entry to 35 years) gay men, in Amsterdam 1984-2002.

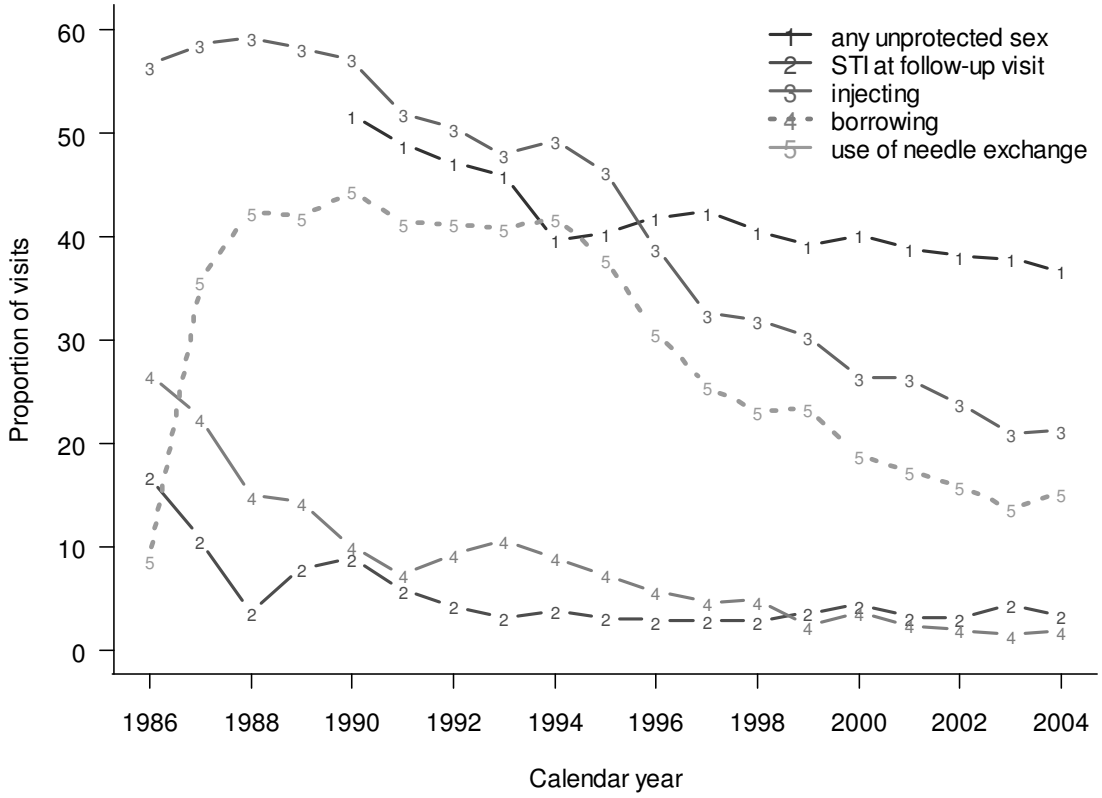

Figure 4. Proportion of visits per calendar year at which injecting and sexual risk behaviour was reported amongst 1315 drug users (DU) who were HIV-negative on ACS entry, 1986-2004.

HCV, HBV and HSV-1 co-infections

In 2006, the retrospective testing for HCV was completed amongst 1276 DU and 1846 DU with at least 2 cohort visits. Amongst ever-injecting DU, the prevalence of HCV antibodies was 85% at study entry, and 31% were co-infected with HIV. All but one of the HCV seroconversions occurred in ever-injecting drug users. The HCV and HIV incidence amongst DU since the start of ACS are shown in Figure 5. The yearly HCV incidence dropped from 28 per 100 person-years in the 1980s to 2 per 100 person years in recent years. The HCV incidence in ever-injecting DU was on average 4.4 times greater than

the HIV incidence, a pattern seen over the entire study period. The HCV prevalence was 1% amongst HM at study entry. All HCV seroconversions occurred in HIV-positive HM. The HCV incidence by HIV serostatus since the start of the ACS is shown in Figure 6.
Hepatitis B virus (HBV) prevalence (i.e., positive test for hepatitis B surface antigen [HbsAg] at study entry) is 50% amongst DU and 60% amongst HM with at least 2 study visits. Herpes simplex virus type 2 (HSV-2) prevalence is 47% amongst young 431 DU (18-30 years) recruited in 1985-1989, but it declined to 14% amongst the 171 young DU recruited in 2000-2004. Data on HBV, herpes simplex virus type 1 (HSV-1) and HSV-2 prevalence and incidence for the total group of HM and IDU is expected to be completed in 2007.

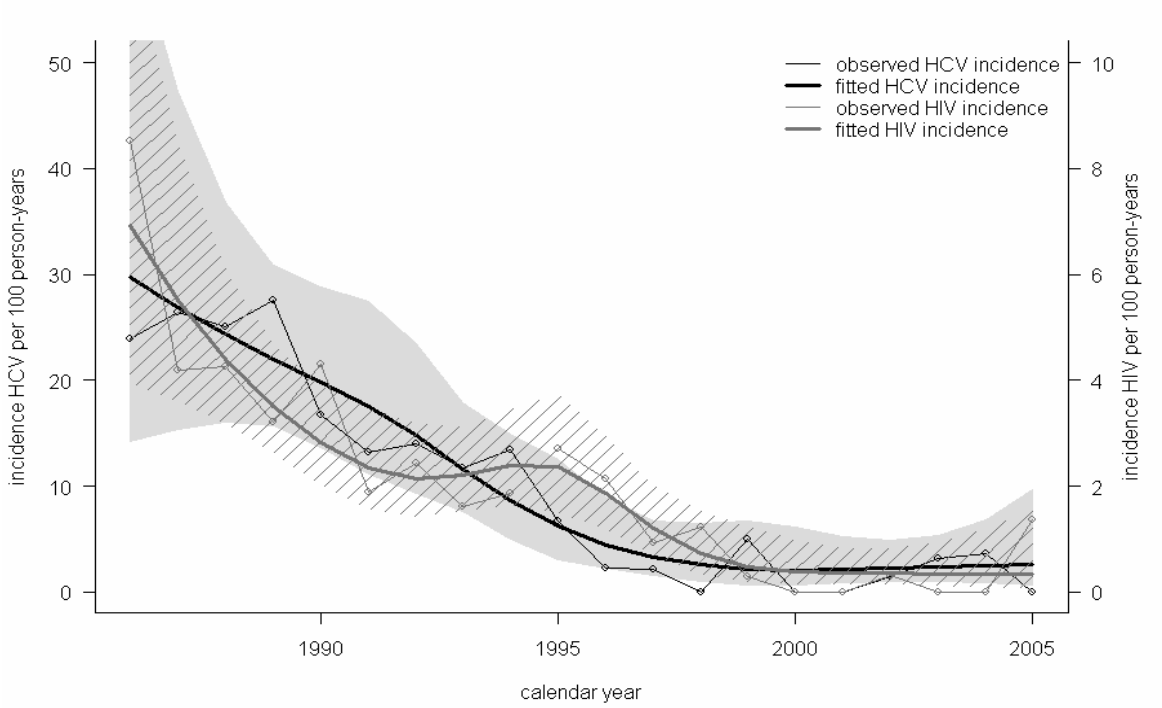

Figure 5. Observed and fitted hepatitis C virus (HCV) (left y-axis) and HIV (right y-axis) incidence curves amongst drug users (DU).

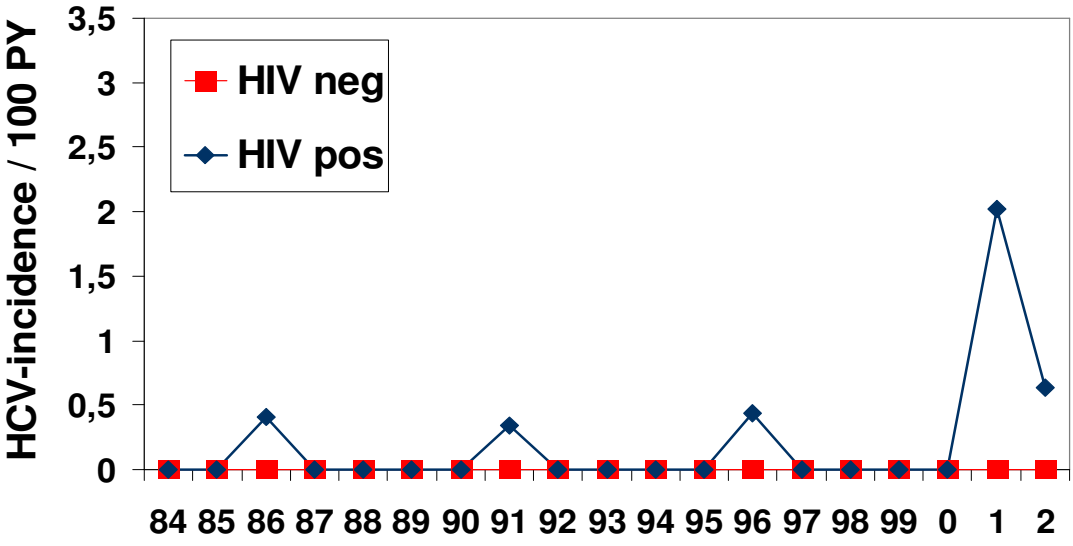

Figure 6. Observed hepatitis C virus (HCV) incidence amongst HIV-negative and HIV-positive homosexual men (HM).

Highly active antiretroviral therapy (HAART) uptake
For the 218 HIV-positive homosexual men visiting the Jan van Goyen Clinic or one of the other HIV treatment centres in the Netherlands in 2005, 177 (81%) received any form of antiretroviral therapy. The viral load was < 400 cells/ml (bDNA) for 157/177 (89%).
Of the 66 HIV-positive drug users who visited the Health Service of Amsterdam in 2006, 38 (58%) received any combination of antiretroviral therapy. Of these, 31 (82%) had an undetectable viral load (Nuclisens < 400 copies/ml) at their latest visit.

Scientfic Highlights

Among HIV negative homosexual men participating in the ACS, low satisfaction with the relationship was associated with more risky unprotected anal intercourse (UAI). High commitment to the relationship was associated with more practice of negotiated sex (i.e., having safe UAI after both partners have tested HIV negative and have reached sexual safety agreements). Using relationship satisfaction, commitment and investment as co-determinants of sexual risk behaviour could prove useful in the development of new HIV-prevention strategies for gay men in steady relationships (Davidovich et al, Journal of applied Social psychology 2006).
Using pooled data form 22 cohorts, including the ACS, we found that in the HAART era compared to the pre-HAART era, the cumulative incidence for all AIDS-related causes of death decreased, but that AIDS opportunistic infections remained the most common cause of death in the HAART era, suggesting that AIDS-related events will continue to be important in the future (Smit el al, AIDS 2006).
Using data from the ACS, we found that drug users receiving HAART are not increasing their injecting and sexual risk behaviour. However, our data suggest that potential HAART start is based on limited drug use. Their early HAART response is similar to homosexual men in the ACS, but drug users started HAART at lower CD4 cell counts and higher viral load levels. Despite the similar response, they never reached the levels of homosexual men and therefore it is likely that therapy is less effective in the long term.
In-depth interviews among young drug users participating in the ACS revealed that motives for injecting and non-injecting can differ widely individually (Witteveen et al, Substance use and Misuse 2006). Some motive are hardly addressed in prevention and should inform new prevention initiatives and programmes (e.g., to enlarge the fear of needles, one could stress the dangers of needle use).
The HIV incidence among drug users in the ACS has substantially declined, accompanied by a reduction in injecting drug use and needle sharing (see figure 4). This decline occurred despite continued sexual risk behaviour. At present new seroconversions are related mainly to unprotected heterosexuals contacts. Therefore, HIV prevention programmes for drug users should pay specific attention to the importance of safe sex practices (Lindenburg et al, AIDS 2006).

Dendritic cells have been proposed to mediate sexual HIV-1 transmission by capturing the virus in the mucosa and subsequently presenting it to T-cells. Most HIV-1 studies have been performed with monocyte-derived dendritic cells. Such in vitro-generated cells may not accurately mimic the myeloid dendritic cells from blood or skin. Plasmacytoid dendritic cells play an important role in the innate immune responses to human viruses, including HIV-1. We determined the influence of these blood-derived cells on HIV-1 infection of T-cells [1]. The myeloid dendritic cells enhance HIV-1 transmission, but the plasmacytoid dendritic cells inhibit HIV-1 replication in T-cells through secretion of interferon-α and a currently unidentified small molecule. This study reports for the first time that the two main types of dendritic cells have opposing roles in HIV-1 infection of T-cells, which should be taken into account when studying the effect of dendritic cells on viral pathogenesis. This publication was accompanied by an editorial review article to highlight this surprise finding.

Plasmacytoid dendritic cells are known to secrete interferon-α as antiviral component, but we demonstrated that these cells produce at least one additional HIV-inhibitory factor that is smaller than 3kDa [1]. This factor is heat-sensitive, but remains to be identified. We could exclude some of the chemokines (SDF-1, RANTES) that block the HIV-1 co-receptor. Alternatively, the inhibitory factor may be an antimicrobial peptide, although we did also exclude β-defensin 1 as candidate. It will be of interest to perform a further search for this natural anti-HIV compound and investigate whether other viruses are inhibited as well. Not only will this broaden our knowledge on the antiviral response of plasmacytoid dendritic cells, but it could also create novel therapeutic options.

Seroreversion, which is defined as a loss of antibody reactivity, is frequently seen in patients infected with hepatitis C virus that clear the virus. In contrast, HIV-1 seroreversion is extremely rare, even in patients treated with antivirals. It is likely that these patients maintain a low level of virus replication that precludes seroreversion. A few cases of HIV-1 seroreversion have been described for patients treated with highly active antiretroviral therapy (HAART) early after virus infection. We investigated anti-HIV antibody levels in 80 patients treated with HAART during chronic HIV-1 infection and who had an undetectable HIV-1 plasma viral load for at least five years [2]. No seroreversion was observed in this group, arguing for ongoing undetectable virus replication under successful therapy.

There are currently 19 antiretroviral drugs, including eight drugs that target the RT enzyme. Characteristic drug resistance mutations have been identified, but it is likely that yet unknown mutations contribute either directly to the drug resistance phenotype or indirectly to compensate for loss-of-function substitutions in the RT enzyme. To identify new drug resistance-associated mutations, we screened 1,322 RT sequences from 1,015 patients; these sequences were available in our genotypic database [3]. We analyzed this RT database with a focus on alternative mutations at RT positions known to be involved in drug resistance. Multiple alternative resistance-associated mutations were identified at RT positions 44, 62, 67, 69, 70, 74, 103, 181, 190, 210 and 219. These initial findings endorse a more extensive search by computerized methods.

Two studies focused on the transmission of drug-resistant HIV-1 variants. Within the CASCADE program that is sponsored by the European Commission, the impact on the natural history of infection was analyzed [4]. Although transmission of drug-resistant HIV-1 variants is associated with a rapid CD4 cell decline soon after infection, no long-term effect on disease progression was observed in the absence of therapy. The impact on subsequent therapy requires a further analysis.

Within the same CASCADE program, we analyzed the evolution of drug-resistant HIV-1 variants upon transmission [5]. Several mutations were stably maintained but others, like 215Tyr/Phe, 70Arg and 184Val in the RT enzyme, evolved, which most likely reflects repair of RT enzyme function and viral fitness. Consistent with this idea, the latter group exhibited a higher CD4+ cell count than the group with non-evolving variants.

We have previously demonstrated that a Lewis X (Le^x)-containing component in human milk binds to DC-SIGN, preventing HIV-1 from interacting with this receptor. Biochemical analysis reveals that the compound is heat-resistant, trypsin sensitive, and larger than 100kDa, indicating a specific glycoprotein as the inhibitory compound. Using mass spectrometry, we identified bile salt-stimulated lipase (BSSL), a Le^x-containing glycoprotein found in human milk, as the inhibitor [6]. Different isoforms of BSSL were present in human milk from different mothers, exhibiting differences in DC-SIGN binding and inhibition of HIV-1 transfer from dendritic cells to T-cells. Elucidation of the precise molecular interaction of BSSL with DC-SIGN may aid in the future design of antimicrobial agents. Furthermore, BSSL will be studied as putative new markers for disease progression.

The 4 human monoclonals antibodies (mAbs) b12, 2G12, 2F5 and 4E10 generally exhibit broadly HIV-neutralizing activity in vitro but their ability to neutralize recently transmitted viruses has not yet been explored in detail. Here, we investigated the neutralization sensitivity of subtype B HIV-1 variants obtained from 4 primary HIV infection cases, and 6 transmission couples (4 homosexual and 2 parenteral) to these broadly neutralizing antibodies and soluble CD4 (sCD4), and also assessed the relation between neutralization sensitivity and the occurrence of mutations in the antibody epitope. HIV-1 variants isolated within the first two months after seroconversion were often resistant to sCD4 and 2G12 (IC_{50} >12.5 □g/ml); for ~90% of the viruses, 2G12 neutralization resistance correlated with the absence of at least one of the 5 N-linked glycosylation sites that are believed to be involved in the make up of the antibody epitope. Moderate resistance was observed for 4E10, whereas neutralization resistance to mAbs b12 and 2F5 was relatively low. Strikingly, resistance to 2F5 and 4E10 neutralization did not correlate with mutations in the respective core epitopes. Although a vast majority (~85%) of the subtype B viruses were sensitive to neutralization by at least 1 of 4 mAbs studied, 4 of 10 patients harboured at least one virus variant that seemed resistant to all 4 antibodies. Our results support efforts to design vaccine antigens that seek to elicit antibodies equivalent to b12, 2G12, 2F5 and 4E10. However, based on our results the identification of additional specificities needs to be pursued.

CXCR4-using (X4) or syncytium inducing (SI) human immunodeficiency virus type 1 (HIV-1) variants evolve from CCR5-using (R5) or non-syncytium inducing (NSI) variants relatively late in the natural course of infection in 50% of HIV-1 subtype B-infected individuals and subsequently coexist with R5 HIV-1 variants. This relatively late appearance of X4 HIV-1 variants is poorly understood. Here we tested the neutralization sensitivity for soluble CD4 (sCD4) and the broadly neutralizing antibodies IgG1b12, 2F5, 4E10, and 2G12 of multiple coexisting clonal R5 and (R5)X4 (combined term for monotropic X4 and dualtropic R5X4 viruses) HIV-1 variants that were obtained at two time points after the first appearance of X4 variants in five participants of the Amsterdam Cohort Studies on HIV-1 infection and AIDS. Recently emerged (R5)X4 viruses were significantly more sensitive to neutralization by the CD4-binding-site-directed agents sCD4 and IgG1b12 than their coexisting R5 viruses. This difference was less pronounced at the later time point. Early (R5)X4 variants from two out of four patients were also highly sensitive to neutralization by autologous serum (50% inhibition at serum dilutions of >200). Late (R5)X4 viruses from these two patients were neutralized at lower serum dilutions, which suggested escape of X4 variants from humoral immunity. Autologous neutralization of coexisting R5 and (R5)X4 variants was not observed in the other patients. In conclusion, the increased neutralization sensitivity of HIV-1 variants during the transition from CCR5 usage to CXCR4 usage may imply an inhibitory role for humoral immunity in HIV-1 phenotype evolution in some patients, thus potentially contributing to the late emergence of X4 variants.

The HLA B*57 allele is overrepresented among human immunodeficiency virus (HIV) infected long-term non-progressors (LTNPs). However, the HLA B57+ allele is also present in up to 11% of HIV-1 infected individuals with a progressive disease course, which is similar to the frequency in the Caucasian population. The association between HLA B57 and an asymptomatic disease course has been attributed to strong CTL responses against epitopes in the viral Gag protein. Moreover, CTL escape mutations in Gag would coincide with viral attenuation, resulting in low viral load despite evasion from immune control. Here we compared HIV-1 variants from HLA B57+ infected progressors and LTNPs for sequence variation in 4 epitopes in Gag and their ability to generate CTL responses against these epitopes and the autologous escape variants were analyzed. Furthermore, we related the replicative capacity of these viruses to the sequence variation in Gag.
LTNPs and progressors were indistinguishable for the magnitude of CD8+ IFNgamma responses directed against peptides with either wild-type or autologous escape mutant amino acid sequences of the epitopes in Gag in ELIspot analysis. Furthermore, the prevalence and dynamics of escape mutations in the 4 Gag epitopes was similar in all HLA B57+ individuals.
Interestingly, HIV-1 variants from HLA B57+ LTNP had much lower replicative capacities than viruses from HLA B57+ progressors which was associated with a higher number of previously described potential compensatory mutations in Gag in progressor viruses. Interestingly, any combination of 2 of these 5 potential compensatory mutations in Gag was significantly associated with disease progression ($p<0.03$). However, differential replication of co-existing progressor viruses could not be explained by the presence or absence of any of these 5 potential compensatory mutations suggesting that additional compensatory mutations in other parts of Gag may exist.

Cytotoxic T lymphocytes (CTL) drive intra-patient evolution of HIV-1 by selecting for escape mutations that interfere with presentation of viral peptides by the host's human leukocyte antigens (HLA) to the immune system or with CTL recognition of the epitope. Transmission and accumulation of CTL escape mutations in the population has suggested a gradual adaptation of HIV-1 to this immune pressure although reversion of CTL escape mutations has been observed as well. These reversions of CTL escape mutations are most likely associated with restoration of viral replication capacity. To more specifically address evolution of HIV-1 upon transmission to a new individual we here performed sequence analysis on HIV-1 variants isolated from 5 therapy naive horizontal HLA-disparate donor recipient pairs from the Amsterdam Cohort Studies on HIV-1 infection. In donor viruses, 20-90% of all differences relative to the HIV-1 subtype B consensus sequence were in predicted CTL epitopes restricted by donor HLA types. In the first weeks after transmission, the majority of these predicted CTL escape mutations in donor HLA restricted epitopes in Gag, Env, and Nef remained present and reversions, both in and outside epitopes, and forward mutations in recipient HLA restricted epitopes, contributed equally to the sequence evolution in this phase of infection. At the end of long-term follow-up of recipients, 43-85% of predicted CTL escape mutations that were transmitted from donor to recipient had reverted to consensus sequence in the recipient. The relatively slow reversion of mutations in CTL epitopes may have implication for vaccine design.

Recently, the tripartite interaction motif 5α (Trim5α) has been identified as an inhibitory factor blocking infection of a broad range of retroviruses in a species specific manner. In particular, HIV-1 replication can be efficiently blocked by Trim5α from Old world monkeys. The viral determinant in HIV-1 for Trim5α is believed to be the cyclophilin A (CyPA) binding region in capsid, and mutations in this region lift the restriction in simian cells. Human Trim5α is also able to inhibit HIV-1 replication *in vitro*, implicating that Trim5α may contribute to host control of HIV-1 replication *in vivo*.
Here we studied the potential role of Trim5α in HIV-1 pathogenesis using Trim5α escape mutations as an indicator for Trim5α mediated inhibition in vivo. Trim5α escape mutants could be demonstrated in 13.7% of the HIV-1 infected individuals, and these variants emerged relatively late in infection after a prolonged asymptomatic phase. Concomitantly, a significant lower plasma viral RNA load was observed 18 months after seroconversion in individuals that developed Trim5α escape variants late in infection as compared to individuals that only carried Trim5α sensitive variants. Our data suggest a role for Trim5α in control of viral burden during the asymptomatic phase of HIV-1 infection.
Both CD4+ T cell help and CD8+ cytotoxic T cell functions are important factors in controlling viral infections. In a large prospective cohort study including all 102 seroconvertors from the Amsterdam Cohort we showed that CD4 helper reponses shortly (12 months) after seroconversion did not relate to sustaining CD8 responses and did not have prognostic value for time to progression to AIDS (Jansen et al, Blood 2006). Our data are in agreement with earlier work that proposed that not CD4 responses determine the viral load but viral load determines the quantity and quality of HIV specific CD4+ T cells (Jansen et al Trend Immunol 2006). Recent analyses in the same seroconvertor cohort showed that also CD8+ T cell responses and the combination of CD4 and CD8 responses have no prognostic value for time to progression to AIDS (I. Schellens manuscript in preparation).

HLA-B27 and B57 are associated with relatively slow progression to AIDS. Previous studies have revealed that CTL responses against Gag epitopes presented by HLA-B57 were significantly higher than those against epitopes presented by HLA-A2. We extended this work by including 75 peptides derived from the entire HIV-1 genome and studied CTL responses towards HIV epitopes restricted through HLA-B27, B57 or A2. CTL responses against a selection of known and predicted HIV epitopes were measured using the IFN-gamma ELISpot assay. Prediction were performed using peptide prediction programs based on MHC-binding, proteasomal cleavage and TAP transport. We found that individuals expressing both HLA-A2 and HLA-B27 responded to significantly more HLA-A2 restricted peptides and with significantly higher magnitude compared to individuals without HLA-B27. We did, however, not find an increase in responses towards HLA-A2 restricted peptides in individuals expressing both HLA-A2 and HLA-B57. In the latter individuals the total CTL response was clearly dominated by HLA-B57 restricted CTL. Viral load was significantly lower in individuals expressing HLA-B27 or B57 compared to individuals expressing HLA-A2 without HLA-B27 or B57, and correlated with the breadth of the HLA-A2 restricted CTL response in the individuals expressing both HLA-A2 and B27. These data suggest that proper suppression of viral load (due to the presence of the 'protective' HLA allele B27) can preserve CTL function, also those restricted by a non-protective HLA allele, whereas the mechanism of protection of HLA-B57 is probably more related to intrinsic features of this HLA molecule. (I. Schellens manuscript in preparation) Of the 22 novel epitopes predicted to be presented by either HLA-B27 or B57, 19 peptides induced IFNgamma production in at least one individual expressing HLA-B27 or B57. The magnitude of CTL responses towards the predicted peptides was similar to the magnitude of responses towards known HLA-B27 or HLA-B57 restricted peptides from the Los Alamos HIV database analyzed in the same HIV-1 infected individuals. These data thereby also show that numerous HIV-1 epitopes may not yet have been identified, and that prediction programs are very powerful tools to identify such novel epitopes.

HIV-infected individuals have a highly increased incidence of (EBV-positive) B cell non-Hodgkin's lymphomas (AIDS-NHL) due to uncontrolled EBV-driven B cell proliferation because of loss of functional EBV-specific CD8+ T cells. We recently observed that a loss of functional EBV-specific CD4+ T cells preceded the CD8+ T cell functional loss. (E. Piriou Blood 2005) EBV-specific CD4+ and CD8+ T-cell responses after HAART showed a decline in T cells specific for lytic EBV protein BZLF1, suggestive of a decreased EBV reactivation rate. In contrast, latent EBV antigen EBNA1-specific T-cell responses were restored to levels comparable with healthy individuals. (E. Piriou J Immunol 2005) Interestingly, EBV load remained unaltered, even after 5 years of therapy. We previously already reported that the absolute level of EBV DNA in PBMC from HIV-infected individuals is not a good predictor of EBV-related lymphoproliferative disease (van Baarle JID 2002) and we showed that a steep increase in EBV DNA load occurred already shortly after HIV infection, in spite of a functional EBV-specific CD8+ T-cell response. Therefore, we hypothesized that increased immune activation

and stimulation of B cells could be a factor that leads to increased EBV DNA levels in the peripheral blood. Interestingly, while changes in EBV load did not correlate with changes in total $CD4^+$ or $CD8^+$ T-cell numbers, changes in EBV load correlated positively with a number of T-cell activation markers ($CD38^+$HLA-DR, Ki67, CD70) on both $CD4^+$ and $CD8^+$ T cells. This indicates that long-term increases in EBV load are more strongly associated with changes in immune activation than with generalized immune deficiency. Furthermore, EBV load before and after HIV-seroconversion correlated significantly. In addition, in 10 individuals included in a longitudinal study on the effects of highly active antiretroviral therapy (E Piriou J Immunol 2005), EBV DNA load early in HIV-infection strongly correlated both with EBV load later in HIV-infection and after 5 years of effective antiretroviral treatment. This shows that, although it remains unclear how HIV affects the biology of EBV, inter-individual differences in EBV DNA load are in great part conserved, even during antiretroviral treatment. Furthermore, when studying T-cell immune activation markers while taking these inter-individual differences into account, it appears that immune activation may well be one the most important factors leading to an increase of EBV DNA load in HIV-infected individuals.(E Piriou, manuscript submitted) These data should encourage the set-up of detailed longitudinal studies of EBV in the setting of predominant co-infections, including determination of numbers of infected cells, niche of the virus (memory B-cells, other B cells, other cells?) and EBV strains present over time. This would provide a better understanding of the co-evolution of this virus in the human lymphoid system, and how both host and pathogen have adapted to allow for an extremely well regulated interaction, even in the presence of major co-infections. Interestingly, we observed high EBV loads also in children under HAART. In these children EBV was not restricted to the B-cell compartment and could also be detected within the CD4 and CD8+ T cells.(V Bekker, J Infect Dis 2006)

CD4+ T cell dynamics
The mechanism of CD4+ T cell depletion in HIV infection is still a major issue of debate. Immune activation and direct effects of HIV infection may contribute. Increased proliferation of CD4 T cells has been interpreted as sign of renewal to replace the cells lost because of HIV infection but we have argued that it may also be immune activation driven and not result in long lived progeny T cells. We set up in vivo labeling of newly produced T cells which is the only way to formally test these quite different hypotheses. In mice a large part of the naïve T-cell pool consists of short-lived recent thymic emigrants (RTE). In humans however, the lifespan and number of RTE are unknown. While ^2H_2O labeling in mice showed high thymic-dependent daily naïve T-cell production, long term up- and down-labeling with ^2H_2O in young human adults revealed a low daily production of naïve T cells. Using mathematical modeling, we estimated human naïve CD4 and CD8 T-cell half-lives of 4.2 and 6.6 years, respectively, while memory CD4 and CD8 T cells had half-lives of 0.4 and 0.7 years. The estimated half-life of recently produced naïve T cells was much longer than these average half-lives. Thus, our data are not only incompatible with a substantial short-lived RTE population in human adults, but also suggest that the few naïve T cells that are newly produced are preferentially incorporated in the peripheral pool.
Similar data obtained in HIV infected patients that were untreated showed increased turnover of memory CD4 and CD8 T cells with a short half life of labeled cells as expected. Interestingly, there was a much higher fraction of newly produced CD4 and CD8 naïve T cells compared to controls, but completely in contrast to what was observed in healthy controls also newly produced naïve RA+CD27+ T cells had a very short life span. Thus, this for the first time unequivocally shows that naïve T cell division does not result in long lived T cells that sustain the naïve T cell pool.

Steering committee: The politburo
In 2006 the Politburo met several times. Twenty-one proposals for use of data and/or samples (serum/PBMCs) were submitted to the politburo: 13 from Sanquin, 7 from UMCU, and 1 from the AHS, All have been approved, some of them after revision.

Publications in 2006 that include ACS data

1. Pillay D, Bhaskaran K, Jurriaans S, Prins M, Masquelier B, Dabis F, Gifford R, Nielsen C, Pedersen C, Balotta C, Rezza G, Ortiz M, de Mendoza C, Kucherer C, Poggensee G, Gill J, Porter K; CASCADE Virology Collaboration. The impact of transmitted drug resistance on the natural history of HIV infection and response to first-line therapy
AIDS 2006;20:21-8

2. Thiébaut R, Jacqmin-Gadda H, Walker S, Sabin C, Prins M, Del Amo J, Porter K, Dabis F, Chêne and the CASCADE Collaboration. Determinants of response to first HAART regimen in antiretroviral-naïve patients with an estimated time since HIV seroconversion
HIV Medicine 2006;7:1-9

3. Smit C, Lindenburg K, Geskus RB, Brinkman K, Coutinho RA, Prins M. Highly active anti retroviral therapy (HAART) among HIV infected drug users: a prospective cohort study of sexual risk and injecting behaviour
Addiction 2006;101:433-40

4. Krol A, Lensen R, Veenstra J, Prins M, Schuitemaker H, Coutinho RA. Impact of CCR5 Δ32/ + Deletion on Herpes Zoster Among HIV-1-Infected Homosexual Men
Eur J Epidemiol. 2006;21:469-73

5. Smit C, Geskus R, Walker S, Sabin C, Coutinho R, Porter K, Prins M. CASCADE Collaboration
Effective therapy has altered the spectrum of cause-specific mortality following HIV seroconversion
AIDS 2006;20:741-9

6. Witteveen E, van Ameijden EJC, Schippers G. Motives for and Against Injecting Drug Use Among Young Adults in Amsterdam: Qualitative Findings and considerations for disease prevention
Substance use and Misuse 2006;7:1001-16

7. Bezemer D, de Ronde A, Prins M, Porter K, Gifford R, Pillay D, Masquelier B, Fleury H, Dabis F, Back N, Jurriaans S, and van der Hoek L on behalf of the CASCADE collaboration. Evolution of transmitted HIV-1 with drug-resistance mutations in the absence of therapy: effects on CD4+ T-cell count and HIV-1 RNA load
Antiviral Therapy 2006;11:173-8

8. Shao W, Lazaryan A, Dorak MT, Penman-Aguilar A, Wilson CM, Margolick JB, Goedert JJ, Prins M, Tang J, Kaslow RA. Cohort- and time-specific associations of CTLA4 genotypes with HIV-1 disease progression. AIDS 2006;20:1583-90

9. Davidovich U, de Wit J, Stroebe W. Relationship characteristics and risk of HIV infection: Rusbult's investment model and sexual risk behavior of gay men in steady relationships
Journal of Applied Social Psychology 2006;36:22-40

10. Lindenburg CE, Krol A, Smit C, Buster MC, Coutinho RA, Prins M
Decline in HIV incidence and injecting, but not in sexual risk behaviour, seen in drug users in Amsterdam: a 19-year prospective cohort study
AIDS 2006;20:1771-5

11. Touloumi G, Pantazis N, Antoniou A, Stirnadel HA, Walker SA, Porter K, on behalf of the CASCADE Collaboration. Highly active antiretroviral therapy interruption: predictors and virological and immunologic consequences
JAIDS 2006;42:554-61

12 Groot F, van Capel TM, Kapsenberg ML, Berkhout B, de Jong EC
Opposing roles of blood myeloid and plasmacytoid dendritic cells in HIV-1 infection of T cells: transmission facilitation versus replication inhibition
Blood 2006;108:1957-1964

13 Cornelissen M, Jurriaans S, Prins JM, Bakker M, van der Kuyl AC
Absence of seroreversion in 80 HAART-treated HIV-1 seropositive patients with at least five-years undetectable plasma HIV-1 viral load
AIDS Research and Therapy 2006;3:3

14 Berkhout B, Back NKT, de Ronde A, Jurriaans S, Bakker M, Parkin NT, van der Hoek L
Identification of alternative amino acid substitutions in drug resistant variants of the HIV-1 reverse transcriptase
AIDS 2006;20:1515-1520

15 Naarding MA, Dirac AM, Ludwig IS, Speijer D, Lindquist S, Vestman EL, Stax MJ, Geijtenbeek TBH, Pollakis G, Hernell O, Paxton WA
Bile salt-stimulated lipase from human milk binds DC-SIGN and inhibits human immunodeficiency virus type 1 transfer to CD4+ T cells
Antimicrobial Agents and Chemotherapy 2006;50:3367-3374

Theses in 2006 that include ACS data

February 24, 2006, Utrecht. U Davidovich. Promotor: W. Stroebe. Co-promotor: Dr. J. de Wit.
Liaisons dangereuses -HIV risk behavior and prevention in steady gay relationships.

September 5, 2006. M.J. Geels. Promotor: Prof dr J. Goudsmit, co-promotor: Dr. W.A. Paxton. Sequence analysis as a tool to determine viral evolution and escape from host immune responses in HIV-1-infected individuals.

October 4, 2006, Amsterdam. G. Tjon. Promotor: Prof. R.A. Coutinho Co-promotor: Dr. S. Bruisten
Molecular epidemiology of hepatitis A in the Netherlands